

# Multidrug-resistant Bacterial Profile and Patterns for Wound Infections in Nongovernmental Hospitals of Jordan

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## Abstract

Globally, multidrug-resistant bacteria affects wound infections, both hospital-acquired infections and community-acquired infections. The main isolates cultured from 607 subjects with wound infections were methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp. [multidrug resistant (MDR)]. Gram-negative bacteria caused most of the infections (67%) compared with gram-positive bacteria. Diabetic patients tend to have wound infections with mixed causative agents compared with non-diabetic patients.

**Keywords:** wounds, skin, diabetic, multidrug, mixed infection

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## INTRODUCTION

The skin or under skin tissue (wound) infections could be deep-seated soft tissue infections. These types of infections include the infections of the skin layer (subcutaneous tissue) and muscle tissue layers. Other deeper infections known as cellulitis target the multilayer of the skin<sup>1</sup>.

Multidrug-resistant (MDR) bacteria that cause wound infection cases are considered one of the most dangerous causative agents. The acute burns and wound infections were found to be related to aerobic gram-positive cocci while the chronic wound infections are related to more complex flora. In addition to *Pseudomonas aeruginosa*, MDR gram-negative bacteria and methicillin-resistant *Staphylococcus aureus* (MRSA) are the most common isolated infectious agents from complicated infected wounds<sup>1-3</sup>. Recent studies have shown that 20–50% of diabetic foot infections are due to MRSA<sup>4,5</sup>.

Antimicrobial resistance in MDR bacteria colonized burn wound infections is a major concern

in intensive care or burns units worldwide<sup>6</sup>. This has resulted in a great threat to efforts against bacterial pathogens<sup>7</sup>. The aim of this study is to determine the rate of antibiotic-resistant bacterial isolates from wound patients at Islamic hospitals, Amman, Jordan.

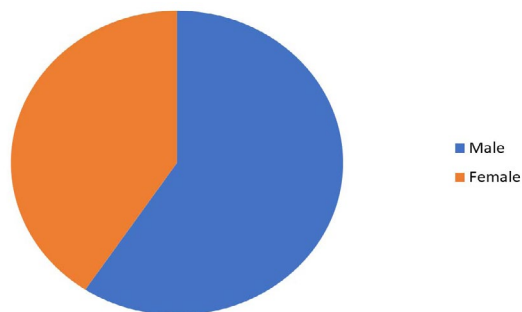
## MATERIALS AND METHODS

### Study design

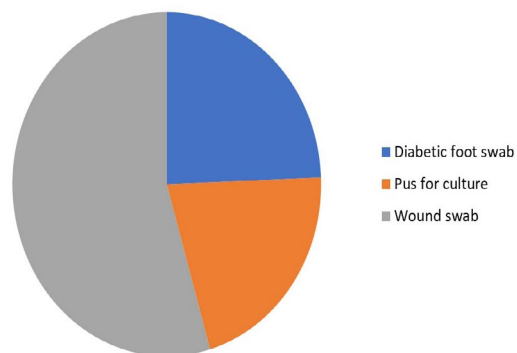
This study was conducted at Islamic hospitals in Amman, Jordan from January 1 to November 11, 2018. In and outpatients from surgery and admitted wards were involved in this study. All the patients who fulfilled the criteria above were consecutively enrolled into the study.

### Sampling and processing

Clinical samples from 607 wound infected patients, including wound swabs 331 (54.4%), pus 128 (21.1%), and diabetic foot swabs 148 (24.4%) were aseptically collected using the appropriate sterile containers. The collected samples were transported to the lab with the appropriate transport media and bacterial



**Fig. 1.** Female and male distribution for diabetic foot swab patients



**Fig. 2.** Study population

**Table 1.** Characteristics of the study population

A- Gender distributions (males and females)			
Type of culture	Male	Female	Total
Numbers of patients for wound swab	207	124	331
Percentage of patients for wound swab	63%	37%	
Numbers of patients for diabetic foot swab	65	83	148
Percentage of patients for diabetic foot swab	44%	56%	
Numbers of patients for pus for culture	88	40	128
Percentage of patients for pus for culture	69%	31%	
Total number	360	247	607
Total percentage	59%	41%	100%

identification to species level was performed using standard procedures including culture and colony characteristics, Gram reaction, and different biochemical analysis.

**Antibiotic sensitivity test**

The antibacterial susceptibility testing was performed using the modified Kirby-Bauer method. Discs of the following antimicrobials were used: ceftriaxone (30 µg), ceftizoxime (30 µg), ceftazidime (30 µg), gentamicin (10 µg), amoxicillin/clavulanate (30 µg), cefuroxime (30 µg), nitrofurantoin (100 µg), cefepime (30 µg), ciprofloxacin (10 µg), ofloxacin (10 µg), pefloxacin (30 µg), clindamycin (2 µg), ampicillin/sulbactam (10/10 µg), imipenem (10 µg), meropenem (10 µg), ertapenem (10 µg), clarithromycin (10 µg), ampicillin (30 µg), Erythromycin (10 µg), ampicillin/

cloxacillin (30 µg), cefixime (5 µg), levofloxacin (10 µg), norfloxacin (10 µg), and metronidazole (5 µg).

**Ethics**

Ethical approval was obtained from the Health Research Ethics Committee of the Ministry of Health.

**Data analysis**

Data analysis was performed using Statistical Package for the Social Sciences (SPSS) version 23 (IBM Corp, 2015). Qualitative data were described as proportions or percentages; cross tabulation was used where necessary. Test of significance for differences for quantitative and categorical variables were performed using the *t*-test and Chi square test, respectively. A *p*-value of < 0.05 was considered significant.

**RESULTS**

**General characteristics**

The total number of patients enrolled in this study was 607; 331 of them were wound infected patients, which forms 54.4% of the total population. Of these, females were 124 (63%) and males were 207 (37%). The patients with diabetic foot ulceration (DFU) were 148, which forms 24.4% of the total population. Among them, 83 (56%) were female patients and 65 (44%) were

**Table 2.** Types of patients and cultures

Types of test	Number of tests	Percentage of total subjects
Diabetic foot swab	148	24.4%
Pus for culture	128	21.1%
Wound swab	331	54.5%
Total	607	100.0%

**Table 3-A.** Growth statistics – Number of growth and non-growth samples of the study population

Type of sample	Number of samples	Percentage of total subjects
Number of growth samples	510	84.0%
Number of non-growth samples	97	16.0%
Total	607	100.0%

**Table 3-C.** Growth statistics – Number of growth and non-growth diabetic foot ulcer samples

Type of sample	Number of samples	Percentage of total subjects
Number of growth samples	130	87.8%
Number of non-growth samples	18	12.2%
Total	148	100.0%

**Table 3-B.** Growth statistics – Number of growth and non-growth wound samples

Type of sample	Number of samples	Percentage of total subjects
Number of growth samples	282	85.2%
Number of non-growth samples	49	14.8%
Total	331	100.0%

**Table 3-D.** Growth statistics – Number of growth and non-growth pus samples

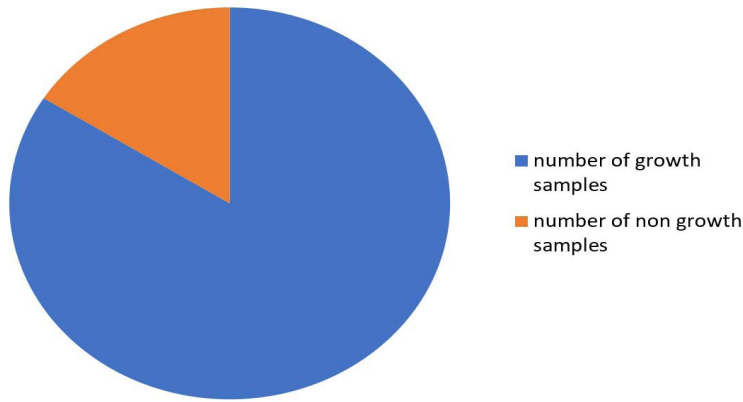
Type of sample	Number of samples	Percentage of total subjects
Number of growth samples	98	76.6%
Number of non-growth samples	30	23.4%
Total	128	100.0%

male patients. The glycemic control for the DFU group was generally poor: 10.5% and more. The pus culture patients were 128, which forms 21.1% of the total population; female patients were 40 (31%) and males were 88 (69%). The overall percentage of females over males is 41/59 (Table 1 & Table 2).

Out of the 607 total samples, 510 (84.0%) samples showed growth. Among wound samples, growth cultures were observed in 282 samples (85.2%) while the non-growth samples were 49

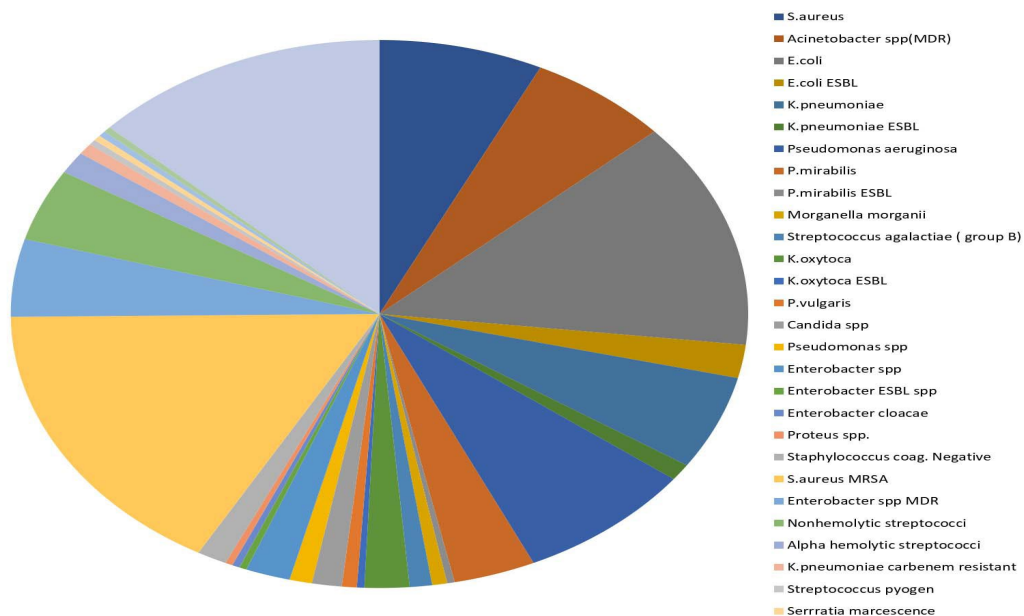
(14.8%). In the case of diabetic foot ulcers, 130 (87.8%) samples showed growth and only 18 (12.2%) samples did not grow. For the pus samples, 98 (76.6%) showed growth and 30 (23.4%) samples did not. (Table 3-A, Table 3-B, Table 3-C, Table 3-D) and Fig. 3.

A total of 30 pathogenic organisms were isolated from the cultivated wound samples, one yeast and 29 types of bacteria. MRSA was the most frequent pathogen followed by *E. coli*, *S. aureus*, *Acinetobacter* spp (MDR), *K. pneumoniae*,



**Fig. 3.** Study population for growth and non-growth.

**Bacterial profile and patterns of infections for wounds cultures**



**Fig. 4.** Bacterial profile and patterns of infection for wounds cultures.

*Enterobacter* spp MDR, and nonhemolytic *Streptococcus* spp. (Table 4-A.1), Descriptive tables (Appendix: Supplementary tables) and Fig. 4.

More than 30% of wound infections were resistant to the antibiotics. The most frequent high

resistant isolates in wound infections were; MRSA, *Acinetobacter* spp. (MDR), *Enterobacter* spp. MDR, *E. coli* ESBL, *K. pneumoniae* ESBL, *K. pneumoniae* carbapenem-resistant, *P. mirabilis* ESBL, *K. oxytoca*

### High resistant drug isolates of wound infection

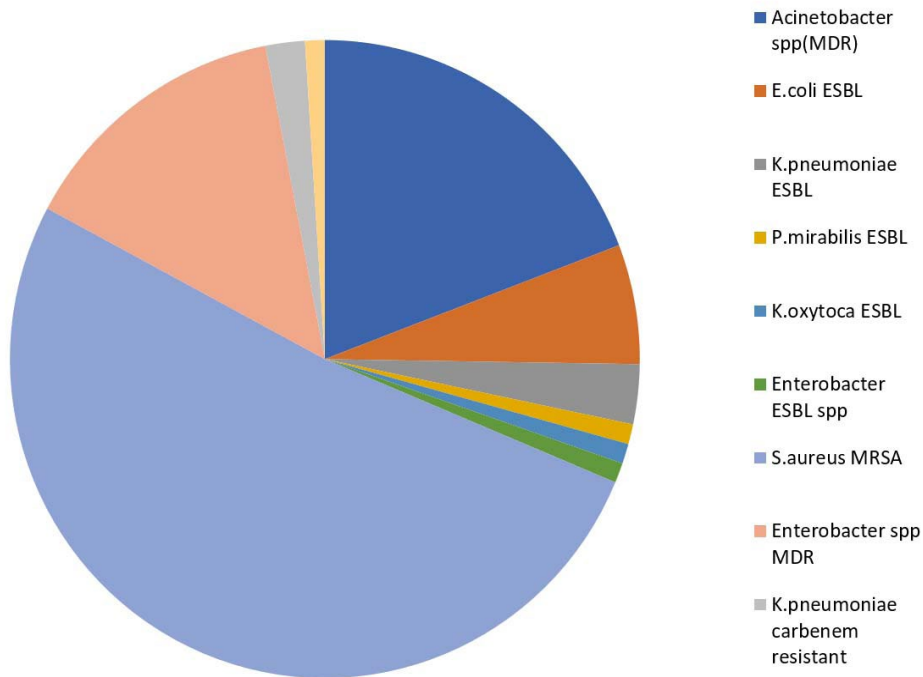


Fig. 5. High drug-resistant isolates of wound infections.

### Multidrug resistant isolates of wound culture

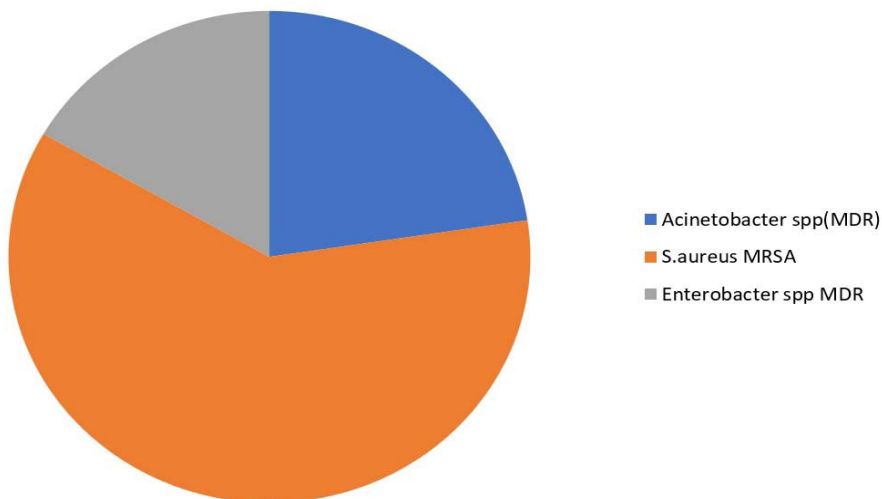


Fig. 6. Multidrug resistant isolates of wound culture

ESBL, *Enterobacter* spp. ESBL, VRE (see Table 4-A.2) and Fig. 5.

The result shows 27.5% of the infections were MDR infections. MRSA was the most aggressive pathogen among the multidrug-resistant isolates (MDR) for wound cultures, which formed more than 51% of MDR agents, followed by *Acinetobacter* spp. (MDR), and then by *Enterobacter* spp MDR (Table 4-A.3) and Fig. 6.

In diabetic foot ulcer swabs, 17 pathogens were isolated. One yeast and 16 types of bacteria.

*S. aureus* was the most frequent pathogen followed by *E. coli*, *Acinetobacter* spp. (MDR), *K. pneumoniae*, *P. aeruginosa*, and *P. mirabilis*. *Enterobacter* spp. ESBL, CoNS, and *Streptococcus viridans* were the less frequent pathogens. *Candida* spp. were recorded as one of the causes of infection in two cases. Nineteen patients had no pathogenic microbes (Table 4-B.1) and Fig. 7.

The most drug-resistant isolates of diabetic foot ulcers were MRSA, followed by *Acinetobacter* spp. (MDR), *P. mirabilis* ESBL, *K. pneumoniae* ESBL, *P. mirabilis*, *P. vulgaris* ESBL, *K. oxytoca* ESBL, *P. mirabilis* ESBL, *P. vulgaris* ESBL, *Enterobacter* spp ESBL, *Staphylococcus coag. Negative*, *Streptococcus agalactiae* (group B), *Morganella morganii*, *Streptococcus group D (enterococcus)*, *P. mirabilis*, *Pseudomonas aeruginosa*, *E. coli*, *Acinetobacter* spp(MDR), *S.aureus* and No pathogenic bacteria.

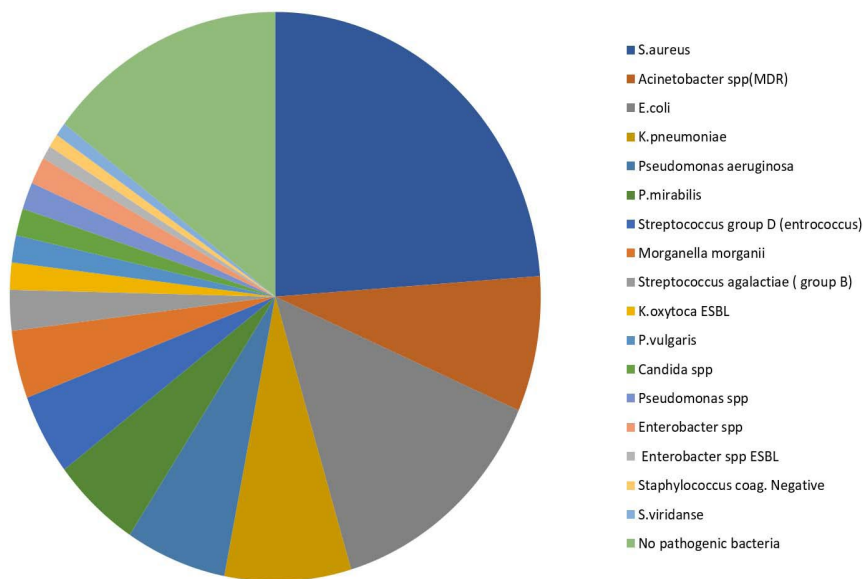


Fig. 7. Diabetic foot ulcer infections

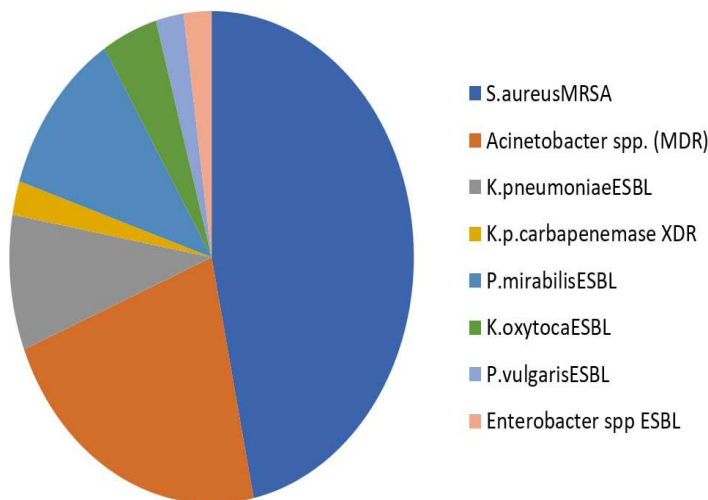


Fig. 8. High drug-resistant isolates of diabetic foot ulcers

*K. pneumoniae* ESBL, *K. oxytoca* ESBL, and *K. pneumoniae* carbapenemase XDR (see Table 4-B.2) and Fig. 8.

A quarter and more of infections in diabetic foot ulcers are caused by multidrug resistant bacteria; MRSA (16.2%) was the most frequent pathogen among these pathogens, followed by *Acinetobacter* spp. (MDR) (7.7%), *K. pneumoniae* carbapenemase XDR (0.8%), and *Enterobacter* spp. ESBL (0.8%) (Table 4-B.2) and Fig. 8.

For the pus samples, 19 pathogens were isolated. *E. coli* (21.4%) was the most frequent pathogen, followed by *S. aureus* (14.0%), and *S. aureus* (MDR) (11.0%), nonhemolytic

streptococci (9.0%), and then the others; *K. pneumoniae*, *S. agalactiae* (group B), *M. morgani*, *E. coli* ESBL, *S. pyogenes*, *K. oxytoca* ESBL, *Acinetobacter* spp. (MDR), VRE, *P. aeruginosa*, *P. mirabilis*, *K. pneumoniae* carbapenemase XDR, *E. coli* carbapenemase XDR, Alpha hemolytic streptococci, and *Acinetobacter* spp. constitute approximately 30% of the total infections (Table 4-C.1) and Fig. 9.

The most drug-resistant isolates of the pus samples were; MRSA, *E. coli* ESBL, *Acinetobacter* spp. (MDR), *K. oxytoca* ESBL, *E. coli* carbapenemase XDR, VRE, *K. pneumoniae* carbapenemase XDR (see Table 4-C.2) & Fig. 10

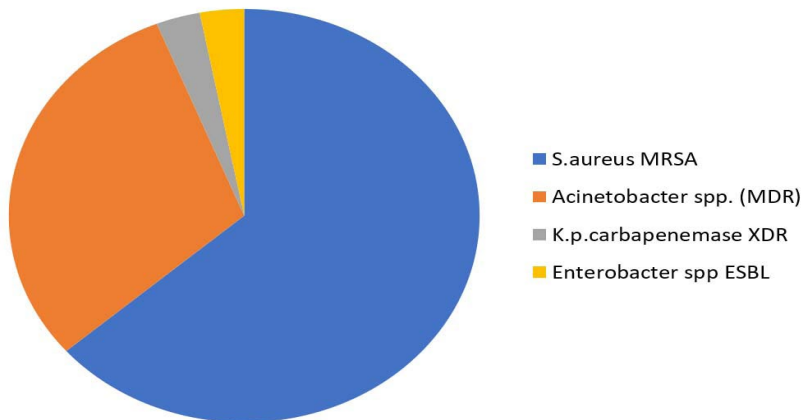


Fig. 9. Multidrug resistant isolates of diabetic foot ulcers

### Infections of pus samples

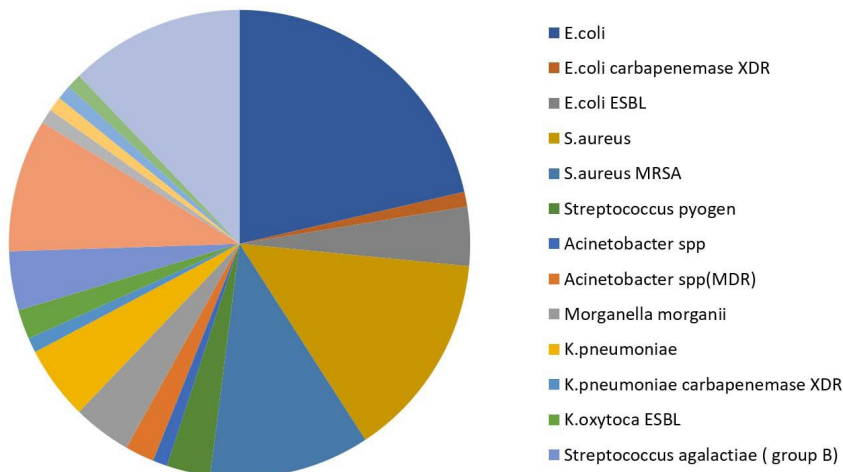


Fig. 10. Pathogenic agents of the pus samples

### High drug resistant and MDR for pus samples

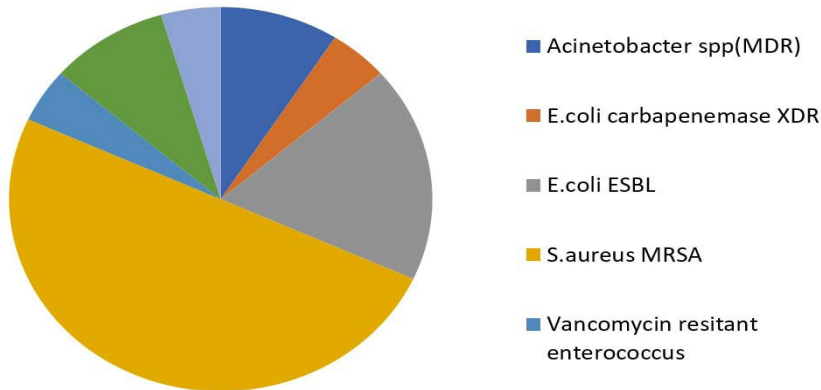


Fig. 11. High drug resistant and MDR isolates of the pus samples

### Multidrug resistant isolates of for pus samples

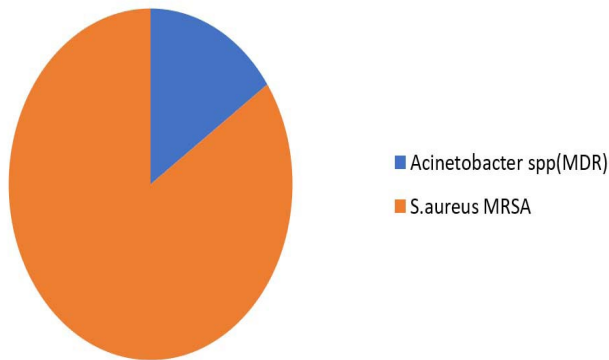


Fig. 12. Multidrug-resistant isolates of the pus samples

### Types of pathogens for the mixed infections of wound infections

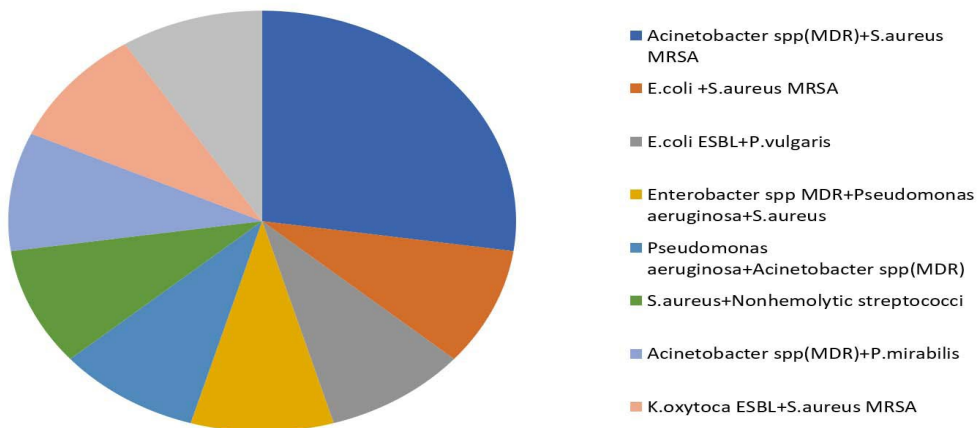


Fig. 13. Types of pathogen combinations for the mixed wound infections



Only 14.3% of infections in the pus samples were caused by multidrug resistant bacteria; MRSA (11.2%) was the main cause of infections and the second cause was *Acinetobacter* spp. (MDR) (3.1%) (Table 4-C.3) and Fig. 11.

The result showed that 3.3% of the wounds were mixed infections caused by more than one bacterium; all of the mixed infections were in diabetic patients (Table 5).

*Acinetobacter* spp. (MDR) and MRSA were the most frequent combination in mixed infections by percent of 27.3% of the total infections of wounds, followed by *E. coli* and MRSA, *E. coli* ESBL and *P. vulgaris*, *Enterobacter* spp. MDR and *P. aeruginosa* and *S. aureus*, *P. aeruginosa* and *Acinetobacter* spp. (MDR), *S. aureus* and nonhemolytic streptococci, *Acinetobacter* spp. (MDR) and *P. mirabilis*, *K. oxytoca* ESBL and MRSA, and MRSA and *K. pneumoniae* (Table 6) and Fig. 12.

**DISCUSSION**

Wound infections are becoming an actual burden of lesions globally. Recently, the World Health Organization reported a catalog of antibiotic-resistant “priority pathogens.” In this catalog was a list of 12 families of bacteria that are the greatest threat to human health. At the top of the list was the most important causative pathogen in wound infection MRSA, which is spreading globally and constitutes the cause of approximately 20% of wound infections. The prevalence of antibiotic-resistant strains of *S. aureus* is increasing at an alarming rate. The highest resistance was recorded against ampicillin and erythromycin (88% each), while resistances against oxacillin, fosfomycin, ceftiofloxacin, and ciprofloxacin were also worrisome<sup>8</sup>.

In this study, the results showed that MRSA accounted for 16.7% of total wound infections. *E. coli* is presented in the list of most common causative agents of wounds, and causes approximately 13.4% of wound infections in Jordan; *P. aeruginosa* accounts for approximately 8% of wound infections in Jordan. *Acinetobacter* spp. (MDR) is the second multidrug-resistant pathogen accounting for 6.2% of the total infections. The other pathogens form less than 50%; *K. pneumoniae*, *Enterobacter* spp. MDR, and nonhemolytic streptococci. The

infectious agents were *E. coli* ESBL, *K. pneumoniae* ESBL, *P. aeruginosa*, *P. mirabilis*, *P. mirabilis* ESBL, *M. morgani*, *S. agalactiae* (group B), *K. oxytoca*, *K. oxytoca* ESBL, *P. vulgaris*, *Candida* spp. (yeast), *Pseudomonas* spp., *Enterobacter* spp.,

**Table 4-A.1.** Bacterial profile and patterns of infection for wound infections

Type of pathogen	Number of infections	Percentage
<i>S. aureus</i>	22	7.2%
<i>Acinetobacter</i> spp. (MDR)	19	6.2%
<i>E. coli</i>	41	13.4%
<i>E. coli</i> ESBL	6	2.0%
<i>K. pneumoniae</i>	17	5.6%
<i>K. pneumoniae</i> ESBL	3	1.0%
<i>P. aeruginosa</i>	24	7.8%
<i>P. mirabilis</i>	11	3.6%
<i>P. mirabilis</i> ESBL	1	0.3%
<i>M. morgani</i>	2	0.7%
<i>S. agalactiae</i> (group B)	3	1.0%
<i>K. oxytoca</i>	6	2.0%
<i>K. oxytoca</i> ESBL	1	0.3%
<i>P. vulgaris</i>	2	0.7%
<i>Candida</i> spp.	4	1.3%
<i>Pseudomonas</i> spp.	3	1.0%
<i>Enterobacter</i> spp.	6	2.0%
<i>Enterobacter</i> spp. ESBL	1	0.3%
<i>E. cloacae</i>	1	0.3%
<i>Proteus</i> spp.	1	0.3%
Coagulase negative <i>Staphylococcus</i> (CoNS)	4	1.3%
Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)	51	16.7%
<i>Enterobacter</i> spp. MDR	14	4.6%
Nonhemolytic streptococci	13	4.2%
Alpha hemolytic streptococci	4	1.3%
<i>K. pneumoniae</i> carbapenem resistant	2	0.7%
<i>S. pyogenes</i>	1	0.3%
<i>Serratia marcescens</i>	1	0.3%
<i>Citrobacter freundii</i>	1	0.3%
Vancomycin resistant <i>Enterococcus</i> spp. (VRE)	1	0.3%
No pathogenic bacteria	40	13.1%
No growth after 48 h	49	
Total	306	100.0%

MDR- multidrug resistant; ESBL- extended spectrum β-lactamase

*Enterobacter* spp. ESBL, *Enterobacter cloacae*, *Proteus* spp., CoNS, Alpha hemolytic streptococci, and *K. pneumoniae* carbapenem of infections (Table 4-A.1) and Fig. 4.<sup>4,9</sup>.

Infection with MDR gram-negative organisms in most cases leads to poorer outcomes in burns, especially in critical cases. These bacteria

and the future horror of multidrug-resistance (MDR), the challenge among Jordanian healthcare providers, and the patients suffering from complications demonstrate the actual burden of infections and the progression of the infections cause the causative bacteria to be highly resistant to wound infections. This study showed that approximately 67% of the infections are caused by gram-negative bacteria while approximately

**Table 4-A.2.** High drug-resistant isolates of wound infections

Type of pathogen	Number of infections	Percentage
<i>Acinetobacter</i> spp (MDR)	19	6.2%
<i>E. coli</i> ESBL	6	2.0%
<i>K. pneumoniae</i> ESBL	3	1.0%
<i>P. mirabilis</i> ESBL	1	0.3%
<i>K. oxytoca</i> ESBL	1	0.3%
<i>Enterobacter</i> ESBL spp	1	0.3%
MRSA	51	16.7%
<i>Enterobacter</i> spp MDR	14	4.6%
<i>K. pneumoniae</i> carbapenem resistant	2	0.7%
VRE	1	0.3%
Total	99	32.0%

**Table 4-B.1.** Diabetic foot ulcer infections

Type of pathogen	Number of infections	Percentage
<i>S. aureus</i>	31	23.8%
<i>Acinetobacter</i> spp. (MDR)	10	7.7%
<i>E. coli</i>	18	13.8%
<i>K. pneumonia</i>	10	7.7%
<i>P. aeruginosa</i>	8	6.2%
<i>P. mirabilis</i>	7	5.4%
<i>Streptococcus</i> group D ( <i>Enterococcus</i> )	6	4.6%
<i>M. morgani</i>	5	3.8%
<i>S. agalactiae</i> ( group B)	3	2.3%
<i>K. oxytoca</i> ESBL	2	1.5%
<i>P. vulgaris</i>	2	1.5%
<i>Candida</i> spp.	2	1.5%
<i>Pseudomonas</i> spp	2	1.5%
<i>Enterobacter</i> spp	2	1.5%
<i>Enterobacter</i> spp ESBL	1	0.8%
CoNS	1	0.8%
<i>S. viridanse</i>	1	0.8%
No pathogenic bacteria	19	14.6%
Total number	130	100.0%

**Table 4-A.3.** Multidrug-resistant isolates of wound culture

Type of pathogen	Number of infections	Percentage
<i>Acinetobacter</i> spp(MDR)	19	6.2%
MRSA	51	16.7%
<i>Enterobacter</i> spp MDR	14	4.6%
Total number	84	27.5%

**Table 4-B.2.** High drug-resistant and MDR isolates of foot ulcer infections

Type of pathogen	Number of infections	Percentage
MRSA	21	16.2%
<i>Acinetobacter</i> spp. (MDR)	10	7.7%
<i>K. pneumoniae</i> ESBL	4	3.1%
<i>K. pneumonia</i> carbapenemase XDR	1	0.8%
<i>P. mirabilis</i> ESBL	5	3.8%
<i>K. oxytoca</i> ESBL	2	1.5%
<i>P. vulgaris</i> ESBL	1	0.8%
<i>Enterobacter</i> spp. ESBL	1	0.8%
Total number	53	40.8%
Total number	98	42.3%

**Table 4-B.3.** Multidrug-resistant isolates of diabetic foot ulcers

Type of pathogen	Number of infections	Percentage of diabetic foot swab infections
MRSA	21	16.2%
<i>Acinetobacter</i> spp. (MDR)	10	7.7%
<i>K. pneumonia</i>	1	0.8%
<i>Enterobacter</i> spp. ESBL	1	0.8%
Total number	33	25.4%

32% are caused by gram-positive bacteria, which is within the global ratios<sup>9,10</sup>.

The highly significant ( $p < 0.001$ ) MDR results are drawing attention to focusing more on handling and taking care of the management and treatment of wounds in Jordan. The risk of developing high drug-resistant isolates of wound was high, approximately 32% of the total infections. The new types of bacteria, especially *E. coli* ESBL, *K. pneumoniae* ESBL, *K. pneumoniae* carbapenem resistant, *P. mirabilis* ESBL, *K. oxytoca* ESBL, *Enterobacter* ESBL spp., and VRE, constituted 8.8% while the actual MDR constituted 23.2% (see Table 4-A.2) and Fig. 5<sup>10</sup>.

The high cost and lack of well-trained multidisciplinary medical personnel, facilities, and standardized management protocols are possible contributory factors. Physicians also have an important role in the prevention, early diagnosis, and management of infections of wounds with multidrug resistant or high resistant microbes for chemotherapies. MDR isolates of foot ulcers were reported though the

patients reported that the ulcers resulted from spontaneous blisters or physicians reported the ulcers as wounds. There is a small percentage of overlap between diabetic foot ulcers and diabetic wounds, therefore, there is a possibility that some of the ulcers may have resulted from unnoticed micro-trauma. Inappropriate footwear might lead to spontaneous blisters; this was found to be the second commonest predisposing event for DFU. In addition, fitting of footwears in patients with peripheral neuropathy may result in foot ulcerations in patients with insensate feet. The use of disordered machines or tools and abnormal weight-bearing in peripheral areas of the foot in patients with peripheral neuropathy could make the foot susceptible to ulceration while wearing shoes.

Self-inflicted burns due to thermal injury resulting from the application of hot compresses to numb feet precipitated two cases of DFU. This might cause ulcerations and wounds or burns and should be taken care of in foot ulceration studies<sup>11</sup>.

**Table 4-C.1.** Pathogenic agents of the pus samples

Type of pathogen	Number of infections	Percentage
<i>E. coli</i>	21	21.4%
<i>E. coli</i> carbapenemase XDR	1	1.0%
<i>E. coli</i> ESBL	4	4.1%
<i>S. aureus</i>	14	14.3%
MRSA	11	11.2%
<i>S. pyogenes</i>	3	3.1%
<i>Acinetobacter</i> spp.	1	1.0%
<i>Acinetobacter</i> spp. (MDR)	2	2.0%
<i>M. morgani</i>	4	4.1%
<i>K. pneumoniae</i>	5	5.1%
<i>K. pneumoniae</i> carbapenemase XDR	1	1.0%
<i>K. oxytoca</i> ESBL	2	2.0%
<i>S. agalactiae</i> (group B)	4	4.1%
Nonhemolytic streptococci	9	9.2%
<i>P. mirabilis</i>	1	1.0%
Alpha hemolytic streptococci	1	1.0%
<i>P. aeruginosa</i>	1	1.0%
VRE	1	1.0%
No pathogenic bacteria	12	12.2%
No growth after 48 h	30	
Total number	98	52.0%

**Table 4-C.2.** High drug-resistant and MDR isolates of the pus samples

Type of pathogen	Number of infections	Percentage
<i>Acinetobacter</i> spp. (MDR)	2	2.0%
<i>E. coli</i> carbapenemase XDR	1	1.0%
<i>E. coli</i> ESBL	4	4.1%
MRSA	11	11.2%
VRE	1	1.0%
<i>K. oxytoca</i> ESBL	2	2.0%
<i>K. pneumoniae</i> carbapenemase XDR	1	1.0%
Total number	22	22.4%

**Table 4-C.3.** Multidrug-resistant isolates of the pus samples

Type of pathogen	Number of infections	Percentage
<i>Acinetobacter</i> spp. (MDR)	3	3.1%
MRSA	11	11.2%
Total number	14	14.3%

**Table 5.** Percentage of mixed wound infections

Number of mixed infections	Percentage of mixed wound infections	Note
11	3.3%	All were diabetic patients

Thus, there is a need to ensure that better-focused education and the determination of the best way to handle and take care of ulcer foot cases on appropriate footwear, foot care, and other harmful practices be intensified among these patients.

**Table 6.** Types of pathogen combinations for the mixed wound infections

Type of pathogen combinations	Number of infections	Percentage
<i>Acinetobacter</i> spp. (MDR) + MRSA	3	27.3%
<i>E. coli</i> + MRSA	1	9.1%
<i>E. coli</i> ESBL + <i>P. vulgaris</i>	1	9.1%
<i>Enterobacter</i> spp. MDR + <i>P. aeruginosa</i> + <i>S. aureus</i>	1	9.1%
<i>P. aeruginosa</i> + <i>Acinetobacter</i> spp. (MDR)	1	9.1%
<i>S. aureus</i> + Nonhemolytic streptococci	1	9.1%
<i>Acinetobacter</i> spp. (MDR) + <i>P. mirabilis</i>	1	9.1%
<i>K. oxytoca</i> ESBL + MRSA	1	9.1%
MRSA + <i>K. pneumoniae</i>	1	9.1%
Total number	11	100.0%

### The bacteriological pattern of diabetic foot ulcers

In the present study, a total of 17 different microorganisms were isolated from the participants, with mixed gram-positive and gram-negative species and the yeast *Candida albicans*; an average of 1:4 gram-positive aerobic bacteria, 4:1 gram-negative aerobic bacteria, and 1:16 yeast gave an overall average of 0.13% (6.7) organisms per case. This is similar to the findings of a study in the USA, which had a larger sample size<sup>12</sup>.

The predominance of gram-negative aerobes has also been reported by field workers and previous studies<sup>13</sup>. These differences could be partly due to changes in the causative organisms occurring over time, and the capability of microbes to develop more resistance to antibiotics. It might also be affected by geographical variations or the types and severity of the infection. The differences in results might be due to the use of a “relatively small number of specimens”, and limited specimen collection techniques, which would fail to exclude superficial or colonizing organisms, poor handling techniques, and poor preservation methods, which might affect the cultivation of anaerobic organisms<sup>14,15</sup>.

### The bacteriological pattern of pus sample isolates

In the present study, a total of 18

different microorganisms were isolated from the participants, most of them (85.7%) were neither multi-resistant nor high resistant bacteria to antibiotics; *E. coli* (21.4%) was the most frequent pathogen followed by *S. aureus* (not multi-resistant) (14%), *S. aureus* (MDR) (11%), nonhemolytic streptococci (9%), and then the others; *K. pneumoniae*, *S. agalactiae* (group B), *M. morgani*, *E. coli* ESBL, *S. pyogenes*, *K. oxytoca* ESBL, *Acinetobacter* spp. (MDR), VRE, *P. aeruginosa*, *P. mirabilis*, *K. pneumoniae* carbapenemase XDR, *E. coli* carbapenemase XDR, Alpha hemolytic streptococci, and *Acinetobacter* spp. constitute approximately 30% of the total infections. This is consistent with the global results with mixed gram-positive and gram-negative species; an average of 1:3 (7/18) gram-positive aerobic bacteria with approximately 44% of the total infections. (Table 4-C.1) and Fig. 9.<sup>16</sup>

The multidrug resistant bacteria in pus isolates represented 14.3% of all the infections and the main causative agents were *Acinetobacter* spp. (MDR), which is prevalent in Jordan as the cause of nosocomial infections. This explains its predominance in pus and chronic infections than MRSA, which is one of the main causes of wound infection worldwide (Table 4-C.3) and Fig. 11.<sup>1,2,12,17</sup>

Fifty-four (8.9%) patients from the study were diabetic, 11 (20%) of them had mixed infections with 3.3% being wound infections (Table 5).

Most (27.3%) of the cases of mixed infections were caused by *Acinetobacter* spp. (MDR) and MRSA, which may be due to hospital-acquired infections and/or the aggressiveness of these bacteria. Mixed infections by more than two microbes were very rare; only one such case was reported in the present study with three mixed causative agents; *Enterobacter* spp. MDR, *P. aeruginosa*, and *S. aureus*. This is an indication of the low chance of multibacterial infections by more than two organisms (Table 6) and Fig. 12.

## CONCLUSION

The global burden from multidrug resistant bacteria affects wound infections, either in hospital-acquired infections or community-acquired infections. The main causative agents of wound infections are MRSA, *E. coli*, *P. aeruginosa*, and *Acinetobacter* spp. (MDR). Gram-negative bacteria caused more than 67% of the infections compared with gram-positive bacteria. Diabetic patients have more predisposition to mixed infections than the non-diabetic patients.

## SUPPLEMENTARY INFORMATION

Supplementary information accompanies this article at <https://doi.org/10.22207/JPAM.15.3.25>

**Additional file:** Additional Table.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

All authors listed have made a substantial,

direct and intellectual contribution to the work, and approved it for publication.

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## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

## ETHICS STATEMENT

This study was carried out in accordance with the recommendations of WHO ethical comity . The protocol was approved by the MOH Scientific Ethical Comity under the protocol number code#MOH REC150029.

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